SPHINGOSINE METABOLISM AND ITS LINK TO PHOSPHOLIPID BIOSYNTHESIS*

Wilhelm STOFFEL

Institut für Physiologische Chemie der Universität zu Köln, D-5 Köln 41, Joseph-Stelzmann-Str. 52, West-Germany

I. Chemistry of long chain sphingolipid bases

Mainly two classes of complex lipids participate in the formation of the lipid phase and beyond this the surface structure of biological membranes. These are the phospholipids, which can be regarded as derivatives of 3-glycerophosphate, and the sphingolipids, which also comprise groups of lipids with rather divergent chemical structures such as ceramide, sphingomyelin, galacto- and glucocerebrosides, sulfatides, the neutral glycosphingolipids and the acidic gangliosides. The lipophilic part of all these compounds is ceramide. These are N-acylated sphingosines. The name sphingosines is used for the whole group of long chain bases with the typical 2-amino-1,3-diol system. There is a structural similarity to the diacylglycerol moiety of the phospholipids. The predominantly saturated alkyl chain of the long chain base attached to carbon atom 3 can be compared with the predominantly saturated fatty acid residues in position 1 and the N-acyl group with the fatty acid in position 2 of the glycerolphosphate backbone. Although a large variety of structurally related long chain bases occurs ubiquitously in bacteria, fungi, plants and animal tissue, sphingosine and dihydrosphingosine are the main long chain bases of sphingolipids of animal cells, whereas phytosphingosine is the predominant representative in plant tissue but can also be found in animal cells. Similar to the fatty acid combinations in different phospholipid species numerous ceramide species are formed by the vast number of fatty acids and different sphingosines. The structure of 25 to 30

* an invited article.

long chain bases has been described in recent years[1, 2], but sphingosine, dihydrosphingosine and phytosphingosine are the main bases. Their chemical structures are presented in figure 1.

Sphingosine, the main long chain base, was described as early as 90 years ago[3], its saturated derivative, dihydrosphingosine, 20 years ago[4] and phytosphingosine 60 years[5] ago. Chemical studies mainly by CARTER et al.[6] unravelled the absolute configuration of dihydrosphingosine as D(+) erythro 2-amino-1,3-dihydroxyoctadecane. Sphingosine possesses a Δ^4-trans double bond[7] (in addition to the two chiral centers of 2S and 3R configuration), and phytosphingosine an additional hydroxy group.

The chirality of C-2 and C-3 leads to four stereoisomers namely the 2S,3R ≡ D(+)erythro, 2R,3S ≡ L(−)erythro, 2R,3R ≡ D(+)threo and 2S,3S ≡ L(−)threo. The synthetic pathway outlined by SHAPIRO[8] and GROB[9] enabled us to synthesize long chain bases ^{3}H and ^{14}C labelled in well defined positions of the molecules[10, 11]. Erythro- and threo-diastereomers can be separated via their N-dichloracetyl derivatives[8], the enantiomeric erythro-forms as ±α-phenylethyl succinamates[11] and the threo isomers as ±glutamates[8, 9]. An important intermediate in the biosynthesis of long chain bases is 3-ketodihydrosphingosine (3-dehydrodihydrosphingosine) and its homologes. We prepared this compound labelled in position 3 with ^{14}C by the oxidation of the N-acetyl derivative according to SNATZKE[12] and hydrolysis of the N-acetyl group in moist ethereal HCl[13]. The hydrochloride of this aminoketone is very stable but under alkaline conditions it cyclizes with aromatization to pyrazine derivatives[13]. We synthesized 2R(−)3-keto-

sphingosine
2S,3R ≡ D(+) erythro
2-amino-1,3-dihydroxy-
octadec-4t-ene

dihydrosphingosine
2S,3R ≡ D(+)erythro
2-amino-1,3-dihydroxy-
octadecane

phytosphingosine
2S,3S,4R ≡ ribo-
2-amino-1,3,4-tri-
hydroxyoctadecane
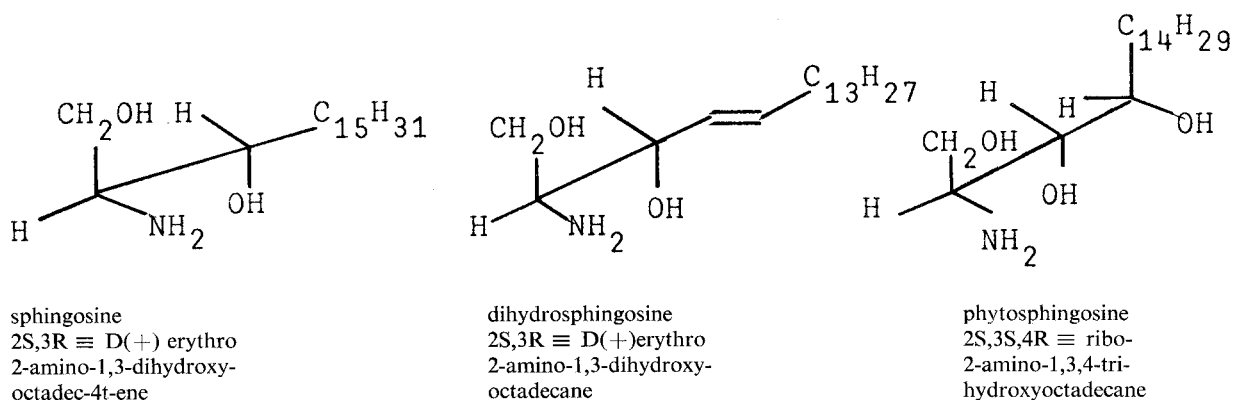
Fig. 1. Chemical structures of main long chain sphingosine bases

dihydrosphingosine ($[\alpha]_{546}^{28} = -26.5° \pm 0.5°$) from 2R,3S L(−) erythro N-acetyl dihydrosphingosine and 2S(+) 3-keto-dihydrosphingosine ($[\alpha]_{546}^{28} = +28° \pm 0.5°$) from 2S,3R D(+)erythro and catalytically reduced natural N-acetyl sphingosine. The racemic compound has also been prepared by others[14, 15].

The elucidation of the biochemical reactions of the sphingosines progressed over years at a slow rate similar to the development of the structural studies. However in recent years their biosynthesis has been unravelled to a large extent and the basic reactions which lead to their degradation have been elucidated, although mechanistic studies regarding the enzymes involved in these reactions await further studies.

In the course of these studies new relationships to phospholipid metabolism became apparent. They will be derived from the results of the experimental work described in the following chapters.

II. Biosynthesis of long chain bases

Early studies *in vivo*[16-20] and *in vitro*[21-24] on the biosynthesis of dihydrosphingosine, sphingosine and phytosphingosine[20] revealed that the 18 carbon atoms of the three long chain bases are derived from serine and acetate or palmitate[18, 19] and palmitaldehyde[21, 22] respectively. Pyridoxalphosphate had been described as an essential cofactor in these reactions, for which BRAUN and SNELL[25] gave the experimental evidence.

In the meantime we had demonstrated that [3-^{14}C] 3-ketodihydrosphingosine is an effective precursor for the formation of dihydrosphingosine and sphingosine in rat liver, which led us to postulate the pyridoxal-phosphate dependent condensation of palmitoyl-CoA and serine with decarboxylation to 3-ketodihydro-sphingosine[26].

WICKERHAM and STODOLA[27-29] described a strain of the yeast Hansenula ciferrii, which produces acetylated dihydrosphingosine and phytosphingosine at a high rate. With the microsomal fraction (100000 × g pellet) of this yeast as enzyme source palmitoyl-CoA and serine condense to 3-ketodihydrosphingosine. This synthase reaction and the subsequent NADPH dependent reduction of the carbonyl group was demonstrated independently and simultaneously in SNELL's[30] and the author's laboratory[31].

The chiral center at C-2 (2S configuration) is derived from L(2S)serine, which is incorporated with retention of configuration, the optical active carbon atom 3 arises from the stereospecific reduction of the carbonyl group. It is catalyzed by a NADPH dependent micro-somal reductase in a stereospecific manner to 2S,3R dihydrosphingosine. The hydride ion from the B-side of the pyridinnucleotide is transferred. The reductase is a lipoprotein[32]. Synthase and reductase exhibit strong chain length specificities with an optimum around 16 to 18 carbon atoms. Studies in vivo with 3-ketoeicosadihydrosphingosine also yielded eicosa-dihydrosphingosine and eicosasphingosine[33]. These biosynthetic reactions are summarized in figure 2.

The reactions described above were confirmed by

148

1) SYNTHASE-REACTION

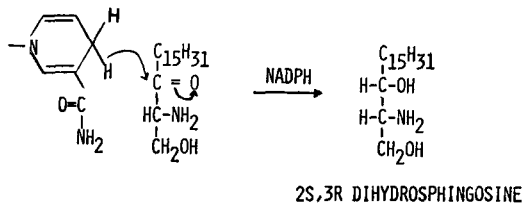2) REDUCTASE-REACTION

2S,3R DIHYDROSPHINGOSINE
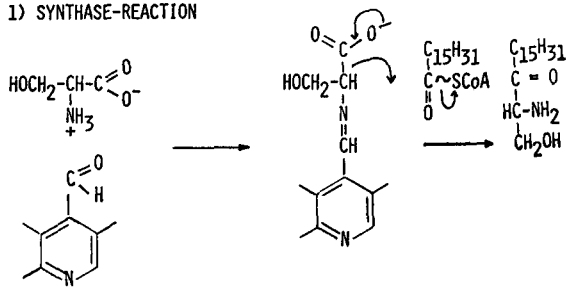
Fig. 2.

analogous experiments with the homologous stearoyl-CoA as acyl donor and serine with mouse brain microsomal fraction[34].

A rather intricate problem arose in our studies on the introduction of the trans double bond in position 4 of sphingosine and of the 4-hydroxy group of phytosphingosine. Our first studies in vivo with 3-ketodihydrosphingosine not only yielded dihydrosphingosine but to a large extent the desaturated sphingosine, both being recovered as components of ceramide and sphingomyelin of rat liver. From chemical reasoning we suggested that 3-ketodihydrosphingosine could be the substrate of the desaturase, in which the hydrogens in α, β-position to the 3-keto group are eliminated[26]. SWEELEY et al.[35], on the basis of their experiments with oyster microsomes came to the conclusion that the desaturation indeed occurs on this intermediate. DI MARI et al.[36] on the other hand claim that 2t-hexadecenoyl-CoA and serine directly condense to 3-ketosphingosine, which is then reduced to sphingo-

sine. If 3-ketodihydrosphingosine were the substrate of the desaturase, then doubly labelled [3-^{14}C; 3-^{3}H] D(+)erythro dihydrosphingosine should loose its ^{3}H isotope. We studied the ^{3}H/^{14}C-ratio of dihydrosphingosine and sphingosine incorporated into sphingomyelin and ceramide after intravenous administration to rats. Our results[37] conclusively demonstrated, that the isotope ratio in sphingosine at carbon atom 3 remained constant. The long chain bases of liver sphingomyelin consisted of more than 80% of [3-^{14}C; 3-^{3}H]sphingosine. 3-Ketodihydrosphingosine could be the substrate under the assumption that the hydrogen atom at C-3 is abstracted stereospecifically with the intermediate formation of the 3-keto group and added again without exchange after the introduction of the double bond. This hypothesis is hard to test and seems to be highly unlikely. Studies on the stereoselective hydrogen elimination by the desaturase and during the hydroxylation of dihydrosphingosine as well were successful. The following experiments regarding the stereochemistry of the desaturation were carried out: Stereospecifically labelled 2S,2R,3S and 3R [^{3}H] palmitic acids mixed with [1-^{14}C]palmitic acid to a definite isotope ratio were injected into brains of myelinating young rats which use the acid for the synthesis of dihydrosphingosine labelled with ^{14}C in position 3 and ^{3}H in position 4 and 5. Hansenula ciferrii utilizes the 2S and 2R-labelled palmitic acids for the synthesis of [3-^{14}C; 4-^{3}H]dihydrosphingosine and phytosphingosine. The isotope ratios of the reaction products sphingosine and phytosphingosine allow conclusions regarding the stereospecificity of the desaturation and hydroxylation. Table 1 demonstrates that 2R and 3S ^{3}H of palmitic acids, which have become the 4R and 5S prochiral ^{3}H of dihydrosphingosine, were eliminated and in yeast the 4R ^{3}H was substituted by the OH group. The results also make clear, that in the enzymatic reactions occurring in rat brain and the yeast Hansenula ciferrii likewise a remarkable kinetic isotope effect is exerted by the hydrogen isotope. The rate limiting reaction is the elimination of the 4R-hydrogen in both cases. This suggests the same mechanism which in one case leads to hydrogen elimination and to hydroxylation in the other. The 5S-hydrogen is eliminated without any isotope effect.

On the basis of these studies we proposed the following stereospecific cis-elimination which leads to the Δ^4-trans double bond of sphingosine:
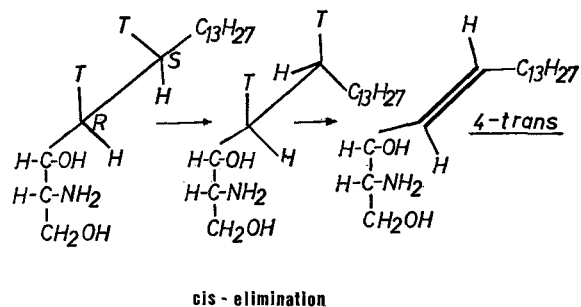
cis - elimination

Fig. 3.

It should be mentioned, that the origin of the hydroxy group of phytosphingosine is not clear at all[38, 39], although dihydrosphingosine has been proven to be the precursor of phytosphingosine[40, 41]. The results of POLITO and SWEELEY[42] are at variance with ours. These authors suggested on the basis of experiments with deuterated palmitic acid that a trans-elimination of the 2R, 3R hydrogens takes place. On the other hand their results agree with ours on the stereoselectivity of the 4R-hydrogen elimination with the formation of phytosphingosine.

The chirality of C-2 and C-3 of dihydrosphingosine

TABLE 1. ISOTOPE RATIOS IN SPHINGOSINE AND DIHYDROSPHINGOSINE OF SPHINGOMYELIN AFTER INTRACEREBRAL INJECTION OF $[1-^{14}C]$ 2S,2R,3S AND 3R PALMITIC ACIDS.

ISOTOPE RATIO $^3H/^{14}C$

IN

SUBSTRATE PALMITIC ACID	PRODUCTS	
	DIHYDROSPHINGOSINE	SPHINGOSINE
$\frac{2R}{[1-^{14}C;2-^3H]}$		
20:1	100:1	1:1
25:1	125:1	3:1
50:1	200:1	1:1
$\frac{2S}{[1-^{14}C;2-^3H]}$		
16:1	14:1	13:1
52:1	50:1	50:1
$\frac{3R}{[1-^{14}C;3-^3H]}$		
54:1	63:1	44:1
25:1	29:1	20:1
$\frac{3S}{[1-^{14}C;3-^3H]}$		
54:1	63:1	13:1
27:1	31:1	6:1
18:1	17:1	5:1

is of the utmost importance for the two reactions mentioned above. We could prove that only the 2S,3R (D(+)erythro) and 2S,3S (L(−)threo) isomers are desaturated to 2S,3R and 2S,3S sphingosine and transformed to 2S,3S,4R and 2S,3R,4R phyto-sphingosine. These results point to the absolute stereospecific requirement of a 2S chiral center at C-2 for the desaturase and the hydroxylation as well[11, 43]. (see Table 2).

III. Degradation of Sphingosine Bases

The chemical synthesis of long chain bases radio-actively labelled in defined positions allowed us to study the degradation of dihydrosphingosine, sphingo-sine and phytosphingosine in experiments in vivo and in vitro. Our first observations[44, 45] were made with $[3-^{14}C]$dihydrosphingosine and $[7,8-^3H_2]$sphingosine. When these two bases were injected into rats, a rapid degradation comparable to the rate of oxidation of $[1-^{14}C]$palmitic acid was measured by the appearance of respiratory $^{14}CO_2$. The bases themselves were mainly recovered as components of liver ceramide, sphingomyelin and to a small extent in cerebroside. About half of the radioactivity was found to be incorporated into the ester lipids (triglycerides and phospholipids), which upon saponification and radio gas-chromatographic identification and quantitation was identified primarily as palmitic acid and a small amount as stearic acid. Dauben degradation localized this radioactivity in C-1 of palmitic and C-3 of stearic acid. Therefore the latter has to be regarded as chain elongation product of the former.

$[1-^3H]$dihydrosphingosine caused a remarkably high incorporation of ^3H-activity into phosphatidyl-ethanolamine, sphingomyelin and to a lesser extent into phosphatidylcholine. Phospholipase C hydrolysis (B. cereus) of the three lipids yielded radioinactive diglycerides, but labelled ceramide, which contained its radioactivity in the long chain base. The total radioactivity released from the two ester phospholipids was present in the aqueous phases as phosphoryl-ethanolamine and phosphorylcholine. In addition the aqueous extract of the liver contained high amounts of phosphorylethanolamine but almost no free ethanol-amine. The results then allowed the conclusion that carbon atoms 1 and 2 are released in toto as ethanol-amine and moreover very likely as phosphorylethanol-amine and C-3 to the terminal CH_3-group as a C_{16},

in the case of eicosadihydrosphingosine[33] as a C_{18} fragment. Sphingosine too always yielded palmitate and never 2t-hexadecenoate as fragment in our experiment in vivo. On the other hand, $[3-^{14}C]$ phytosphingosine prepared biosynthetically from di-hydrosphingosine by Hansenula cif. released $[1-^{14}C]$ α-hydroxypalmitate in the yeast. It is degraded to pentadecanoic acid in the mammalian cell[46].

Our results have been confirmed in several laboratories[47-51]. The occurrence of the C_2-fragment ethanolamine as phosphorylated product induced studies on the degradation in vitro[52]. The degradation proved to be ATP dependent. This suggested that a 1-phosphate ester is formed initially. In fact, when synthetic $[1-^3H; 3-^{14}C]$dihydrosphingosine 1-phosphate was incubated with rat liver particulate fraction (mitochondria and microsomes) it was cleaved into phosphorylethanolamine and palmitaldehyde.

Sphingosine 1-phosphate lyase (aldolase) is bound to the endoplasmic reticulum and mitochondrial membrane. The enzyme is present in all organs of the rat but also in non-mammalian tissue e.g. Hansenula cif. The enzyme requires pyridoxalphosphate, it can be inhibited by deoxypyridoxinphosphate. SH-alkylating reagents (iodoacetamide, N-ethylmalein imid) inhibit the enzyme strongly[52, 53].

Thrombocytes contain a highly active kinase. We used this enzyme to prepare biosynthetically the sphingosine- and phytosphingosine 1-phosphate esters[54, 55] on a preparative scale. These compounds like dihydrosphingosine 1-phosphate are cleaved to phosphorylethanolamine and 2t-hexadecenal and 2-hydroxy palmitaldehyde respectively[56].

Recently, we succeeded in the separation of the antipodes of racemic erythro and threo dihydro-sphingosine. The phosphorylation and lyase reactions were studied with regard to their stereospecificity with these antipodes[11]. Experiments in vivo, which were confirmed by in vitro studies with the purified kinase[57], demonstrated that the four antipodes of the threo and erythro series are substrates of the kinase, although their Km values differed. The lyase on the other hand proved to be highly specific. Only the 2S,3R (D(+)erythro) isomer as its 1-phosphate ester is the substrate[11]. The stereochemical requirements and properties of the lyase allow the formulation of the following mechanism.

The anionic phosphate group is being bound to site 1 which allows the formation of a Schiff base with
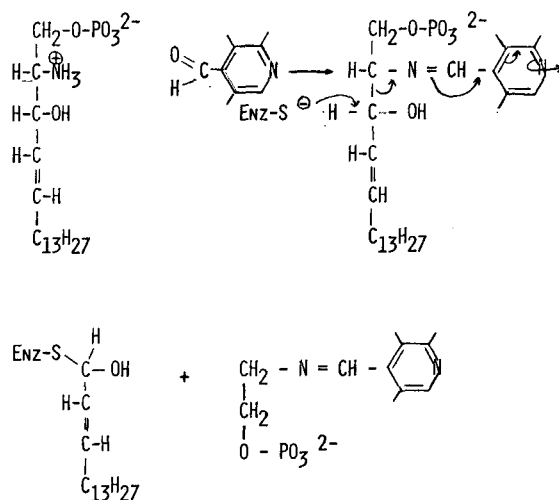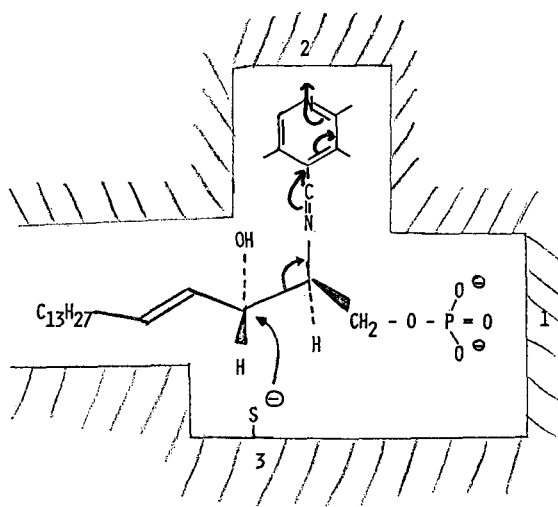

Fig. 4A.

Fig. 4B.

pyridoxalphosphate at site 2 provided C-2 has the correct 2S-configuration. The nucleophilic attack of a sulfhydryl anion (site 3) at C-3 with the formation of a thiohemiacetal requires a 3R (erythro) configuration. The alkane chain beyond C-4 seems to be of little influence. Neither the 4,5-double bond nor a hydroxy group in position 4 nor the length of the chain influence the reaction rate dramatically. Investigation with homologous dihydrosphingosine bases between C_4 and C_{20} showed that the short chain homologues are excreted partly unchanged but they are like the long chain homologues also degraded.

151

The ATP dependent kinase has been studied in several laboratories[58-60]. KEENAN's results on the in vitro degradation of dihydrosphingosine agree well with our's[52, 57].

The studies on the stereospecificity of reactions of the four optically active dihydrosphingosines revealed other interesting results[11]: all four bases are acylated with long chain fatty acids to ceramide regardless their chirality. Only the 2S,3R (D(+)erythro) and 2S,3S (L(−)threo) isomers with identical chirality of C-2 are incorporated into sphingomyelin, cerebroside and ceramidepolyhexosides. It is remarkable that their ceramide part in both cases contains the unsaturated long chain bases.

Regarding the origin of phytosphingosine in animal tissue the hydration of sphingosine has been suggested. However experiments in this laboratory with $[5,6-^3H_2]$ and $[U-^{14}C]$phytosphingosine proved that the orally administered base is largely degraded according to the mechanism put forward in the preceding chapter via 2-hydroxy palmitate to pentadecanoic acid. An appreciable amount however is absorbed by the intest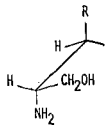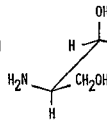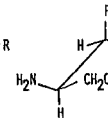i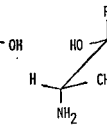nal mucosa and is recovered from rat liver and kidney sphingomyelin and cerebrosides[61]. In none of our numerous experiments regarding the metabolism of $[^3H]$ and $[^{14}C]$dihydrosphingosine and sphingosine were we able to detect any transformation into phytosphingosine which can easily be detected and its structure determined by radio thin-layer chromatography and radio gas-liquid chromatography of the respective pentadecanal after periodate oxidation. Therefore, we suggest that phytosphingosine occurring in sphingolipids of animal tissue is derived from exogenous (plant) sources.

IV. Interrelationship of sphingosine and phospholipid synthesis

The period of myelination is characterized, with regard to lipid biosynthesis, by two simultaneously occurring enhanced synthetic processes namely synthesis of sphingolipids and plasmalogens. In addition the correlation of the sphingolipid and plasmalogenic phosphatidylethanolamine e.g. in myelin and, in general, in brain is remarkable. These facts led to further studies of the metabolic fate of the degradation products of dihydrosphingosine and sphingosine, phosphorylethanolamine, palmitaldehyde and 2t-hexadecenal.

The first indication of the importance of phosphorylethanolamine, which is released in the lyase reaction from the 1-phosphorylated long chain bases, for the de novo synthesis of phosphatidylethanolamine, came from isotope experiments in vivo with $[1-^3H]$ labelled dihydrosphingosine. The ^3H-activity, to a very large extent, was located in the base of phosphatidylethanolamine and phosphatidylcholine[62]. When the $[1-^3H]$ long chain bases or their 1-phosphoryl derivatives were compared with $[3-^{14}C]$ serine, for their precursor function in phosphatidylethanolamine biosynthesis in rat liver, ethanolamine and choline were predominantly labelled with ^3H[63]. The incorporation of C-2 and C-3 of serine was surprisingly low even if serine was used in a five to six fold molar excess over the long chain bases. The incorporation rate of long chain bases into sphingolipids in animal cells in general is rather slow. The concentration of free bases in the cell is negligible. Any excess of sphingosines, such as after intake of sphingolipids with the diet, is immediately phosphorylated and degraded. The following interpretation of these results

Table 2.

	2S,3R (=D(+)ERYTHRO)	2R,3S (=L(−)ERYTHRO)	2R,3R (=D(+)THREO)	2S,3S (=L(−)THREO)
	%	%	%	%
CERAMIDE	11.6	55.7	44.3	89.7
BASE	74.2 (64% D 18:1⁴ᵀ; 36% D 18:0)	100 (97% D 18:0; 3% D 18:1⁴ᵀ)	100 (THREO D 18:0)	100 (98.2% THREO D 18:0; 1.8% THREO D 18:1⁴ᵀ)
FATTY ACIDS	25.8 (82% 16:0; 18% 18:0)	0	0	0
SPHINGOMYELIN	38.5	0	0	10.3
BASE	90 (80% D 18:1⁴ᵀ; 20% D 18:0)	−	−	100 (69% THREO D 18:0; 31% THREO D18:1⁴ᵀ)
FATTY ACIDS	10 (90% 16:0; 10% 18:0)	−	−	0
CEREBROSIDE	3.3	0	0	0
1-PHOSPHATE ESTER	TRACES	20.7 (D 18:0)	43.8 (THREO D 18:0)	0
ESTER FATTY ACIDS	46.6 (79% 16:0; 21% 18:0)	0	0	0
SUBSTRATE	0	23.6	11.9	0

R = C₁₅H₃₁-   D 18:0 = SPHINGANINE   D 18:1⁴ᵀ = SPHINGENINE

may be suggested: The long chain bases are amphophilic compounds and as such will be integrated into the lipid phase of the membranes. As mentioned before, their degradation occurs by enzymes of the endoplasmic reticulum in the neighbourhood of the enzymes of phospholipid biosynthesis operating at a high rate.

Serine enters several metabolic pathways. The experiments of NEMER et al.[64] indicate that 2% of serine are utilized for lipid synthesis, among others, of sphingosine bases, phosphatidylethanolamine and glycerol. 37% pass into the extracellular space, 47% enter the C_1-metabolism, 7% into glucogenesis and 5% are utilized for protein synthesis.

This quantitative study underlines the importance of phosphorylethanolamine, derived from the degradation of long chain bases of endogenous and exogenous sources, as precursor of the hydrophilic group of the main phospholipids.

Additional precursors for phospholipids come from the alkyl chain of the sphingosines. 2t-Hexadecenal derived from sphingosine is first reduced to palmitaldehyde by a soluble 2t-alkenal reductase, which is NADPH dependent[65]. Palmitaldehyde is then either oxidized to palmitate or reduced to cetylalcohol (hexadecanol) by a NADH dependent alcohol dehydrogenase which is specific for long chain aldehydes. Cetylalcohol is a precursor for the biosynthesis of the alkylether and further of the alkenylether linkage of plasmalogens. This reaction sequence was demonstrated in this laboratory by the following experiments: [3-³H]dihydrosphingosine was injected intracerebrally to young rats (10–22 days) during their myelination period. The plasmalogens of the brain tissue were analyzed for the distribution of the ³H-activity. It was found to be exclusively incorporated into the alkyl- and vinylether group of plasmalogens, which released palmitaldehyde as dimethylacetal after acid methanolysis. Therefore part of the palmitaldehyde from the [3-³H]labelled base is reutilized for plasmalogen biosynthesis. When [3-³H]dihydrosphingosine or the [1-³H]palmitaldehyde, released from it, are compared with [1-¹⁴C]palmitate as precursors of plasmalogen biosynthesis, the former two are by far superior to palmitate. We studied the precursor product relationship in vivo of [1-¹⁴C; 1-³H] palmitaldehyde and cetylalcohol, 1-[1-¹⁴C]hexadecyl-3-glycerophosphate, 1-[1-¹⁴C]hexadecyl-2-[³H]linoloyl-3-glycerophosphate, 1-[1-¹⁴C]hexadecyl-3-glycero-

phosphorylethanolamine and 1-[1-¹⁴C]hexadecyl-2-[³H]linoloyl-3-glycerophosphorylethanolamine for the plasmalogen biosynthesis. We obtained strong evidence for the sequence:

Palmitaldehyde → cetylalcohol → 1-hexadecyl-3-glycerophosphate → 1-hexadec-1'-enyl-3-glycerophosphate ----→ plasmalogenic phosphatidylethanolamine[66, 67]. Snyder et al.[68] gave evidence for the precursor function of long chain alcohols in alkyl glycerol ether synthesis in Ascites tumor cells and tumors of the preputial gland. These results form a link with our results regarding the precursor function of palmitaldehyde liberated from the long chain bases. In vitro studies with the aforementioned labelled potential intermediates should establish the sequence also in cell free systems.

We are well aware that the results regarding the substrate of the desaturation to the vinylether bond reported here are at variance with those of other authors[69-71]. SNYDER et al.[69] and PALTAUF[70] postulate that the desaturation takes place at the alkyl-acyl-glycerophosphorylethanolamine level, and DEBUCH[71] claims that alkyl-3-glycerophosphorylethanolamine is the immediate precursor of plasmalogens.
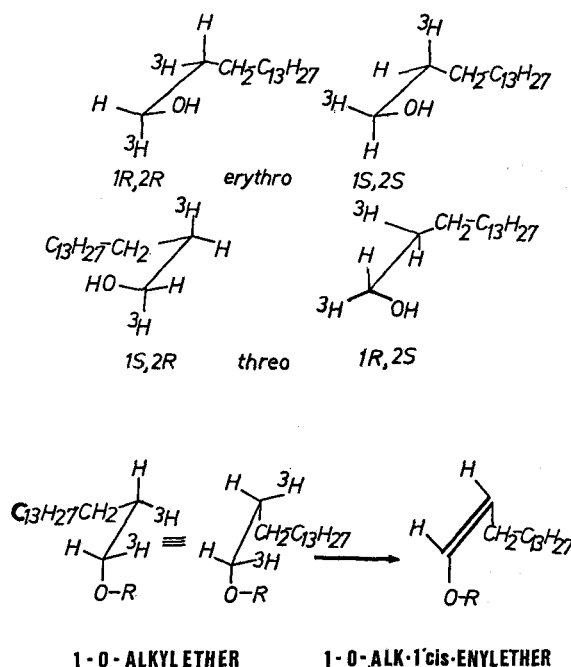

Fig. 5.

Finally, our studies on the stereospecificity of the introduction of the cis-double bond of the vinylether should be described in this context. For this purpose, [1-^{3}H]palmitaldehyde was reduced with alcohol dehydrogenase and cold ethanol to [1-^{3}H] 1S hexadecanol. The tosyl ester of this alcohol was inverted to the 1R isomer of [1-^{3}H]hexadecanol[72]. The corresponding 2R and 2S[2-^{3}H]hexadecanols were obtained by LiAlH$_4$-reduction of the corresponding methyl palmitates, the synthesis of which has been described before[73]. ^{3}H/^{14}C-ratios were adjusted with [1-^{14}C]hexadecanol. When these four alcohols were injected intracerebrally and the plasmalogens analyzed for their radioactive palmitaldehydes it became obvious that the 1S, 2S ^{3}H label was eliminated exclusively in a highly stereospecific cis-elimination, yielding the cis-double bond of the vinylether linkage of plasmalogen. The mechanism is visualized in figure 5.

The mechanism of the alkylether formation with the primary alcohol group of glycerol is still a matter of concern and intensive studies in many laboratories and awaits elucidation by experiments in vitro.

References

[1] K. A. Karlsson, (1970) Lipids 5, 878.
[2] W. Stoffel, (1971) Annual Review of Biochemistry 40, 57.
[3] J. L. W. Thudichum, (1884) "A Treatise on the Chemical Constitution of the Brain", Bailliere, Tindall and Cox London, p. 149. Facsimile Edition Archon Books, Connecticut 1962.
[4] H. E. Carter, W. D. Celmer, W. E. M. Lands, K. L. Mueller, H. H. Tomizawa, (1954) J. Biol. Chem. 206, 613.
[5] P. A. Levene and W. A. Jacobs, (1912) J. Biol. Chem. 11, 547.
[6] H. E. Carter, D. Shapiro and J. B. Harrison, (1953) J. Amer. Chem. Soc. 75, 1007.
[7] K. Mislow, (1952) J. Amer. Chem. Soc. 74, 5155.
[8] D. Shapiro, H. Segal and H. M. Flowers, (1958) J. Amer. Chem. Soc. 80, 1194.
[9] C. A. Grob and E. F. Jenny, (1952) Helvetica Chimica Acta 35, 2106; 34, 2249 (1951).
[10] W. Stoffel and G. Sticht, (1967) Hoppe-Seyler's Z. für Physiol. Chem. 348, 1561.
[11] W. Stoffel and K. Bister, (1973) Hoppe-Seyler's Z. für Physiol. Chem. 354 in press.
[12] G. Snatzke, (1961) Chem. Berichte 94, 729.
[13] G. Sticht, D. LeKim and W. Stoffel, (1972) Chem. Phys. Lipids 8, 10.
[14] P. B. Mendershausen and C. C. Sweeley, (1969) Biochemistry 8, 2633.
[15] R. O. Brady, S. J. Di Mari and S. S. Snell, (1969) J. Biol. Chem. 244, 491.
[16] I. Zabin and J. F. Mead, (1953) J. Biol. Chem. 205, 271.
[17] I. Zabin and J. F. Mead, (1954) J. Biol. Chem. 211, 87.
[18] D. B. Sprinson and A. Coulon, (1954) J. Biol. Chem. 207, 585.
[19] B. Weiss, (1963) J. Biol. Chem. 238, 1953.
[20] M. Greene, T. Kaneshiro and J. H. Law, (1965) Biochim. Biophys. Acta 98, 582.
[21] R. O. Brady and G. J. Koval, (1958) J. biol. Chem. 233, 26.
[22] R. O. Brady, J. V. Formica and G. J. Koval, (1958) J. biol. Chem. 233, 1072.
[23] Y. Fujino and I. Zabin, (1962) J. biol. Chem. 237, 2069.
[24] Y. Fujino, (1964) Agr. Biol. Chem. 28, 807.
[25] P. E. Braun and E. E. Snell, (1967) Proc. Nat. Acad. Sci. USA 58, 298.
[26] W. Stoffel, D. LeKim and G. Sticht, (1967) Hoppe-Seyler's Z. Physiol. Chem. 348, 1570.
[27] L. J. Wickerham and F. H. Stodola, (1960) J. Bacteriol. 80, 484.
[28] F. H. Stodola and L. J. Wickerham, (1960) J. biol. Chem. 235, 2584.
[29] F. H. Stodola, L. J. Wickerham, C. R. Scholfield and H. J. Dutton, (1962) Arch. Biochem. Biophys. 98, 176.
[30] P. E. Braun and E. E. Snell, (1968) J. biol. Chem. 243, 3775.
[31] W. Stoffel, D. LeKim and G. Sticht, (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 664.
[32] W. Stoffel, D. LeKim and G. Sticht, (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1637.
[33] W. Stoffel and A. Scheid, (1969) Hoppe-Seyler's Z. Physiol. Chem. 350, 1593.
[34] P. E. Braun, P. Morell and N. S. Radin, (1970) J. biol. Chem. 245, 335.
[35] R. K. Hammond and C. C. Sweeley, (1972) unpublished.
[36] S. J. Di Mari, R. N. Brady and E. E. Snell, (1971) Arch. Biochem. Biophys. 143, 553.
[37] W. Stoffel, G. Assmann and K. Bister, (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1531.
[38] S. R. Thorpe and C. C. Sweeley, (1967) Biochemistry 6, 887.
[39] W. Stoffel and E. Binczek, (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1065.
[40] W. Stoffel, G. Sticht and D. LeKim, (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1149.
[41] B. Weiss and R. L. Stiller, (1967) J. biol. Chem. 242, 2903.
[42] A. J. Polito and C. C. Sweeley, (1971) J. biol. Chem. 246, 4178.
[43] W. Stoffel and E. Binczek, unpublished.
[44] W. Stoffel and G. Sticht, (1967) Hoppe-Seyler's Z. Physiol. Chem. 348, 941.
[45] W. Stoffel and G. Sticht, (1967) Hoppe-Sey'lers Z. Physiol. Chem. 348, 1345.
[46] W. Stoffel and G. Sticht, (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1149.
[47] Y. Barenholz and S. Gatt, (1967) Biochem. Biophys. Res. Commun. 27, 319.

[48] Y. Barenholz and S. Gatt, (1968) Biochemistry 7, 2603.

[49] K.A. Karlsson, B. E. Samuelsson and G. O. Steen, (1967) Acta Chem. Scand. 21, 2566.

[50] R. W. Keenan and K. Okabe, (1968) Biochemistry 7, 2696.

[51] A. Nilsson, (1968) Biochim. Biophys. Acta 176, 339.

[52] W. Stoffel, G. Sticht and D. LeKim, (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1745.

[53] W. Stoffel, D. LeKim and G. Sticht, (1969) Hoppe-Seyler's Z. Physiol. Chem. 350, 1233.

[54] W. Stoffel, G. Assmann and E. Binczek, (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 635.

[55] W. Stoffel, G. Heimann and B. Hellenbroich, (1973) Hoppe-Seyler's Z. Physiol. Chem. in press.

[56] W. Stoffel and G. Assmann, (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 1041.

[57] W. Stoffel, B. Hellenbroich and G. Heimann, (1973) Hoppe-Seyler's Z. Physiol. Chem. in press.

[58] R. W. Keenan and B. Haegelin, (1969) Biochem. Biophys. Res. Commun. 37, 888.

[59] C. B. Hirschberg, A. Kisic and G. J. Schroepfer, Jr., (1970) J. biol Chem. 245, 3084.

[60] R. W. Keenan and A. Maxam, (1969) Biochim. Biophys. Acta 176, 348.

[61] G. Assmann and W. Stoffel, (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 971.

[62] W. Stoffel, G. Sticht and D. LeKim, (1969) Hoppe-Seyler's Z. Physiol. Chem. 350, 63.

[63] R. Henning and W. Stoffel, (1969) Hoppe-Seyler's Z. Physiol. Chem. 350, 827.

[64] M. J. Nemer, E. M. Wise, Jr., F. M. Washington and D. Elwyn, (1960) J. biol. Chem. 235, 2063.

[65] W. Stoffel and G. Assmann, (1973) Hoppe-Seyler's Z. Physiol. Chem. in preparation.

[66] W. Stoffel, D. LeKim and G. Heyn, (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 875.

[67] W. Stoffel and G. Heyn, (1973) Hoppe-Seyler's Z. Physiol. Chem. in preparation.

[68] F. Snyder, R. L. Wykle and B. Malone, (1969) Biochem. Biophys. Res. Commun. 34, 315.

[69] R. L. Wykle, M. L. Blank, B. Malone and F. Snyder, (1972) J. biol. Chem. 247, 5442.

[70] F. Paltauf, (1972) Biochim. Biophys. Acta 260, 352.

[71] H. Fürniss and H. Debuch, (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 1377.

[72] W. Stoffel and D. LeKim, (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 501.

[73] G. J. Schroepfer, Jr. and K. Bloch, (1965) J. biol. Chem. 240, 54.